ToF-SIMS Characterization of Silk Fibroin and Polypyrrole Composite Actuators

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Abstract

Biocompatible materials capable of controlled actuation under biologically relevant conditions are in high demand for use in a number of biomedical applications. Recently, we demonstrated that a composite material composed of silk biopolymer and the conducting polymer polypyrrole can bend under an applied voltage using a simple bilayer device. Here, further characterization of these bilayer actuators using time-of-flight secondary ion mass spectrometry is presented. The roles of different electrolyte components and factors affecting device performance and stability are clarified. Results

of this study are discussed in the context of strategies for optimization of device performance.

Keywords

ToF-SIMS, actuator, polypyrrole, biocomposite, bilayer, artificial muscle

1. Introduction

Conducting polymers are attractive materials for biomedical devices because they can undergo electrically controlled volume changes, have the ability to interface with biological systems *in vivo* and *in vitro*, and can generate stress in excess of human muscle [1]. Conducting polymer bilayer actuators function through the migration of ions into and out of the polymer layer, causing swelling or shrinking of the conducting polymer. The volume change of the film causes the support material to be pushed or pulled, creating biomimetic movement. The ability of polypyrrole (PPy) to be operated at biologically relevant voltages and to be continuously cycled between positive and negative voltages [1] makes it a good candidate for use in biomedical applications such as steerable catheters [2], tissue engineering scaffolds [3], and biocompatible valves [4], among others. However, PPy is brittle and insoluble, making it difficult to fabricate into devices without the use of a support material. Typical films created with PPy are therefore supported with a metal backing to improve conductivity and lend mechanical support. Unfortunately, metal supports can cause tissue damage in biological systems, and are known to delaminate from the PPy [5,6].

Previously, we reported a method of fabricating PPy actuators that do not require a metal backing [7]. In this work, silk fibroin, a low-cost biopolymer, was used as the

support material. To facilitate integration of PPy into the silk support, sulfanilic acid groups were attached to the tyrosine amino acids through a diazonium coupling reaction, covalently adding negatively charged sulfonate ions to the silk. When pyrrole was polymerized in its cationic form in the presence of these films, the electrostatic attraction between the anionic sulfonate ions and the cationic pyrrole created an interpenetrating network (IPN) of silk and PPy (Figure 1). SEM micrographs revealed that an IPN between silk and PPy is formed in the top ~20 μm of a ~50 μm silk substrate (Figure 1). The high mechanical strength of silk fibroin and its ability to be used *in vivo* with minimal tissue damage make it a desirable substrate for biocompatible actuators [8]. The IPN structure allows the PPy to retain advantageous mechanical properties of the silk, while preventing the delamination that is common in metal supported actuators.

Depending on the chemical composition of the polymer and electrolyte, PPy actuators can display characteristics during actuation consistent with the migration of anions, cations, or both [5,9]. The devices reported here were found to exhibit characteristics consistent with anion exchange only, where films contract under reduction and expand under oxidation [7]. In this mechanism, counter-anions and accompanying solvent molecules are expelled when the cationic PPy is reduced to its neutral form, resulting in a contracting motion. Upon oxidation, the anions are re-inserted, resulting in expansion or stiffening of the film. Actuation experiments were carried out in a biologically relevant electrolyte that contains both phosphate and chloride anions. The role that each ion plays must be understood in order to optimize performance. Therefore, the ability to characterize changes in the concentrations of ions

present in the IPN through individual phases of actuation will provide a better understanding of how each anion contributes to, or inhibits, the actuation and/or degradation processes.

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a powerful analysis technique that has been used to characterize the molecular composition of conducting polymers [10-12] and composite materials [13,14]. PPy films and composite materials have been extensively characterized using static mode ToF-SIMS. During static ToF-SIMS, the surface of the sample is bombarded with ions to fragment the material. These molecular fragments are then analyzed by time-of-flight mass spectrometry. The information obtained allows for the characterization of the molecular composition of the material. In addition to the static mode of ToF-SIMS, conducting polymer films have been characterized using depth profiling [15]. In this technique, a cluster ion beam is used to sputter through the material, allowing for the film to be characterized as a function of depth within the film. Cluster ion beams have been used for depth profiling of amino acids [16], biopolymers [17], and ionic conducting polymer actuators [18-20]. While a variety of cluster ion sputtering beams has been developed, argon cluster ion beams have shown a steady sputtering rate through organic materials [16,21] and reduced damage when compared to C_{60}^+ sputter beams [16,22,23].

Here, the characterization of silk/PPy biocomposite actuator films using ToF-SIMS depth profiling is presented. This technique provides an opportunity to profile concentrations of specific phases of the biocomposite material, as well as specific components of the electrolyte. The composition of the IPN and the profiles of various electrolyte components were studied as a function of the actuation history of the device,

depth from the film surface and proximity to the contact electrode. The findings are discussed in the context of the actuation mechanism of these devices and opportunities for further improvements in device stability and performance.

2. Materials and Methods

2.1 Preparing Silk Fibroin Films.

Cocoons from *Bombyx mori* silkworms were cut into pieces and boiled in aqueous sodium carbonate solution (Na_2CO_3 , 0.02 M) for 1 h. The fibers were transferred to boiling DI water for 10 min, rinsed for an additional 10 min in room temperature DI water three times, and then left to dry in a fume hood overnight. The isolated fibers were combined with lithium bromide (9 M) to make a 20% w/v solution, covered with foil, and placed in a 60 °C oven for 1 h. The resulting solution was transferred to hydrated dialysis tubing (Fisherbrand 3500 MWCO) and dialyzed against DI water for at least 24 h, changing the water after 1 h, 4 h, 12 h, and 16 h of dialysis. The pH of the silk solution was adjusted to pH 7 by dialyzing against dilute aqueous hydrochloric acid (pH 4). The solution was then concentrated by dialyzing against aqueous polyethylene glycol (PEG, 10% w/v) overnight. The final solution was passed through a 5 μm syringe filter to remove any remaining particulate. The concentration of the solution was calculated as dry weight per volume in g/mL. The solution was adjusted to 7% w/v by diluting with nanopure water, and stored in the refrigerator.

Silk sheets were cast using aqueous silk solution (7% w/v). The solution was cast on a 3 x 4 cm area on a silicone baking sheet (from a commercial retailer) to give a porous morphology to the face of the film in contact with silicone [7]. The solutions were

left to dry in a fume hood for 24 h. The dried films and sheets were soaked in ethanol (70% v/v) for 24 h and then slowly transitioned to nanopure water for storage. Specifically, 3 to 5 mL of water was added to the Petri dish every 15 min for at least 90 min. The sheets were then removed from solution and stored in nanopure water.

2.2 Acid-Modification of Solid Silk Films.

Silk sheets were placed in a Petri dish, covered with borate buffer (8 mL, 100 mM, pH 9.0-9.5), and placed on ice. Sulfanilic acid (34 mg, 0.2 mmol) and p-toluenesulfonic acid monohydrate (pTSA, 152 mg, 0.8 mmol) were dissolved in DI water (2 mL) and placed on ice for 10 min. Sodium nitrite (4 M NaNO₂, 34 μ L, 0.14 mmol) was mixed into this solution and returned to ice for 15 min. The reaction mixture was added to the Petri dish containing a free floating silk sheet, and left to react for 40 min on ice. The reaction mixture was decanted and the sheet was rinsed 2-3x with nanopure water before being stored in nanopure water.

2.3 Chemical Polymerization of Pyrrole and Deposition on Silk Films.

As previously described [7], to ensure selective PPy deposition on one side of the sheet, frames of double-sided tape were created by placing tape in a rectangle at the bottom of a Petri dish. Acid-modified sheets were then stretched over the frame and adhered to the tape. The porous face of the sheet is left exposed in the frame to allow for the deposition of PPy onto only that face of the silk sheet. An aqueous solution of pTSA (18 mL, 6 mM) was transferred to the Petri dish containing the acid-modified sheet. Pyrrole (72 μ L, 1.0 mmol) was then added to the Petri dish and mixed. An aqueous solution of iron (III) chloride (FeCl₃) (2 mL, 0.52 M) was immediately added

and the solution was again mixed. The reaction was left to sit for 2 h. After the reaction period, the sheet was rinsed 2-3x with water before being cut out of its frame and stored in nanopure water. To prepare devices, 3 x 10 mm strips were cut from the parent sheet using a razor blade. SEM imaging of our devices showed that the thickness of our films were between 40 to 60 μm thick, with IPN layer having a thickness of 12 to 20 μm .

2.4 Actuation Experiments.

Actuation experiments were carried out using a two-electrode electrochemical cell in phosphate buffered saline solution (PBS, 0.1 M phosphate, 0.15 M NaCl, pH 7.2), utilizing the bilayer silk-PPy film as the working electrode and a 25 x 25 mm platinum mesh as the combined reference/counter electrode. Electrical contact was made to the films by mechanically fixing the end of the film to a gold foil using an inert plastic clamp. The clamp was then lowered so that the electrical contact was just above the solution interface. Actuator films (3 mm x 10 mm x 0.05 mm) were positioned with the PPy-modified side facing the platinum electrode. All potentials were applied using a Keithley 2400 Sourcemeter controlled by LabView. To measure the stress generated during actuation, the conditions for free-end bending actuations described above were replicated while films were held taught in an Aurora Scientific 300C-LR Dual-Mode Muscle Lever System equipped with a 600A digital controller.

The actuation process used in this study consists of a 'cure' phase (a 1 Hz square wave with amplitude ± 2.0 V applied to the sample for 50 cycles and ending on an oxidation cycle), followed by an actuation phase consisting of a 1/120 Hz square wave (± 2.0 V) applied for a specified number of cycles. Literature reports have suggested that the initial applied voltage cycle of typical PPy actuators results in

irreversible volume changes, and further shifts in the oxidation and reduction potentials have been observed over the first 20 cycles [9,24]. A cure phase of actuation is therefore commonly carried out to account for these possible changes, without inducing degradation by applying a large potential for an extended period of time. Following actuation, devices were immediately removed from electrolyte and stored between glass microscope slides until further testing with ToF-SIMS. Some redistribution of ions could occur during this period of time or during the ToF-SIMS measurement itself, potentially leading to non-representative ion profiles as measured by ToF-SIMS. However, the area under the profile curve, serving as a proxy for overall concentration, will not be affected by this redistribution. Therefore, the conclusions of this study rely on profiles when dealing with non-mobile species only. Samples were not rinsed prior to testing in order to preserve the established ion profiles. Although it is possible that some salts could remain adhered to the surface of the sample and affect the resulting ion profiles, comparison of pristine samples with samples immersed in electrolyte prior to testing showed no significant differences.

2.5 Characterization.

ToF-SIMS measurements were performed at Environmental Molecular Sciences Laboratory (EMSL), located at Pacific Northwest National Laboratory. Dual beam depth profiling mode was employed, using a 20 keV argon cluster ion beam (nominal cluster size = Ar_{1500}^+) as the sputtering ion beam and a 25 keV Bi_3^+ beam as the analysis beam. The beams were operated in interlaced mode, with signals being sampled every 3.278 seconds. The sputter region was set to a 200 x 200 μm^2 raster size and the analysis beam was aligned at the center of the sputter area, with a scan area of 50 x 50

μm^2 . Sputtering through the IPN with the Ar cluster ion beam took between 120 and 210 seconds. Sheet resistivity of the PPy-modified side of bilayer films was measured in ambient atmosphere with a four point resistivity probe (Signatone SP4-40045TBY tip) powered by a Keithley 2400 Sourcemeter, and calculated by Lucas Pro4 software. To obtain cross-sectional SEM images, the strips were fractured while held under liquid nitrogen then mounted on vertical stubs using carbon tape. Micrographs were taken using a Vega TS 5136MM SEM with a 15 kV source.

2.6 ToF-SIMS Data Analysis

The peaks at $m/z = 24$, 25 , and 26 in the negative ion mass spectrum are assigned in our analyses to the ions C_2^- , C_2H^- , and CN^- , respectively, and indicate the presence of PPy in the IPN in agreement with previous studies [10,14]. The acid-modified tyrosine amino acid fragments into CNO^- , benzenesulfonic acid radical and CN^- , observed as peaks at $m/z = 42$, 156 , and 24 , respectively, allowing identification of the region of the film containing acid-modified silk. The peak observed at $m/z = 171$ corresponds to the pTSA that is incorporated during PPy synthesis. Interlaced operation during TOF-SIMS depth profiling causes reduced signal strength when sampling insulating material, resulting in a steep drop in H^- intensity at the transition between the IPN and the insulating silk support (Figure 2). Accordingly, this signal drop is used to identify the time at which the ion beam reaches the interface between the IPN and the silk substrate.

In this study, all depth profiling is reported as a fraction of the IPN depth. The fractional depth is set to zero at the air/IPN interface and unity at the IPN/silk interface. In this way, variations in sampling time and IPN thickness are accounted for in each

analysis. The raw ion signal intensities recorded during ToF-SIMS characterization can vary between samples and detected ions. Therefore, where indicated, raw signals are corrected to the C^- ion signal by dividing each signal by the C^- signal recorded at the same time. C^- was chosen because of its relatively stable signal strength throughout the IPN, and because the carbon signal is expected to be relatively constant throughout the sample. The resulting corrected data then allows direct comparison of intensities between samples for the same ion fragment. In some cases, the data was also normalized by dividing all data by the peak value, eliminating the ability to compare signal intensities but better enabling comparisons between profiles of different secondary ions across an individual sample.

3. Results and Discussion

In order to investigate the morphology and makeup of the IPN, the relative signals corresponding to CN^- (indicating PPy or the acid-modified layer as detailed above), CNO^- (indicating the acid-modified layer), C_2^- (indicating PPy) and the benzenesulfonic acid fragment were studied as a function of fractional depth as described in section 2.6 (Figure 3). Both the CNO^- and benzenesulfonic acid intensities increase as a function of fractional depth. The benzenesulfonic acid fragment comes from the azo-bonded group added during acid modification, and is indicative of the presence of silk. The same fragmentation pattern has been observed in dyes with molecular structures similar to the acid-modified tyrosine [25,26]. The rising signal intensity of these two indicators of silk therefore suggests an increase in silk density as a function of fractional depth. This observation was expected, given that the silk films are cast on a rough silicone surface to intentionally make the surface more porous. In

contrast, decreasing signal intensities for both CN^- and C_2^- ions are observed as fractional depth increases. Both acid-modified silk and PPy can contribute to the CN^- signal when fragmented, while the C_2^- is associated only with the fragmentation of PPy. The results are consistent with microscopy images that show the amount of PPy decreases with fractional depth (Figure 1 and previous work [7]), likely due to the decreased silk density close to the IPN/air interface.

Depth profiling of our devices was carried out after various stages in the actuation process as described in section 2.4. For reference, representative actuation data, including current response and the corresponding stress generated during each cycle, are presented in the supplemental information provided. In these devices, the film is generating stress and expanding/stiffening during oxidation with the incorporation of anions, and relaxing during reduction with the expulsion of anions. For the depth profiling, the applied bias across each device was terminated either after the cure phase, or at the end of an oxidation (positive applied bias) or reduction (negative applied bias) cycle prior to depth profiling. One of the profiles studied was the dopant pTSA. ToF-SIMS analysis of the pTSA-doped PPy films showed that the entire pTSA molecule can be observed at $m/z = 171$, in agreement with literature [10,14]. Dopants are typically incorporated during polymer deposition, and serve to increase the conductivity and overall device performance. Draining of the dopant during actuation is a concern for device performance [27]. In this study, the non-normalized depth profiles of pTSA in a non-actuated device, in a device following the cure phase, and after the eighth oxidation and reduction cycle were measured (Figure 4). While the overall signals fluctuate, no evidence of an overall decrease in concentration was observed.

Therefore, we are able to conclude that draining of pTSA during actuation is not a significant effect in our devices.

In addition to measuring whether the dopant concentration changes during actuation, we are interested in understanding the roles of the various components of our electrolyte in the actuation or degradation processes. The electrolyte used in this study is PBS, chosen to simulate actuation performance in a biologically relevant environment, and contains sodium cations and both phosphate and chloride anions. Characterizing changes in the concentrations of these anions in the device during actuation should provide a better understanding of the role that each plays. Chloride ions were detected at $m/z = 35$ and 37 in ratios similar to the natural abundances of these isotopes. The normalized signals from $m/z = 35$ and 37 were added together for ease of reporting purposes and labeled as Cl^- . A peak for PO_4^- was present at $m/z = 95$ but was not large enough to allow for comparison between samples. Because of the limited signal strength of PO_4^- , the PO_2^- and PO_3^- ions detected at $m/z = 63$ and 79 and were combined for reporting purposes and labeled as PO_4^- . Figures 5a and 5b show non-normalized profiles of the indicators of Cl^- and PO_4^- from samples at various stages of actuation, respectively. Chloride anions in the non-actuated samples are expected due to the use of FeCl_3 as an oxidant during pyrrole polymerization. After the cure phase, an increase in the concentrations of both Cl^- and PO_4^- are observed. This suggests that incorporation of both anions occurs during this stage of actuation. The larger relative change in phosphate at this stage indicates that it is likely the primary species responsible for the large bending angle observed during the cure phase (see reference 7). Following the cure phase, the Cl^- concentration increased after each

successive oxidation phase more than it decreased after each reduction phase, resulting in a buildup of chloride throughout the actuation process as shown in Figure 5a. PO_4^- concentrations also increased significantly during the cure phase as shown in Figure 5b, however the fluctuation in signals seen following the cure phase was within error. We therefore conclude that PO_4^- is not ejected from the film in significant amounts during any stage in the actuation process, and further that there appears to be no overall buildup of PO_4^- during actuation.

The observation that certain anions enter the films preferentially, and in some cases build up over subsequent actuation cycles, has significant implications for device performance. Specifically, one might anticipate that actuation performance will decrease dramatically following the first voltage application, as a buildup of anions will prevent further anion movement and therefore deflection. This is consistent with the observation that the large deflection observed in devices during the initial cure phase is not repeated in subsequent actuation cycles. The fact that expansion during oxidation occurs much faster than the relaxation during reduction can possibly also be explained by the relative rates at which Cl^- anions enter and leave the film. These results suggest that a modified square wave potential might be beneficial, in which the reduction potential is held longer, or has a larger magnitude than the oxidation potential in order to extract the remaining anions from the film. The exploration of modified waveforms is a subject of ongoing research.

Finally, we were interested in exploring how the anion profiles change as a function of the distance from the point at which electrical contact is made. The deflection of the devices during actuation is observed as a hinging motion, where the pivot point is

located just below the electrolyte/air interface, indicating that the actuation primarily occurs at the point closest to the electrical contact. In order to explore this effect, devices were sampled at various distances from the electrode, and characterized by ToF-SIMS to show the difference in anion concentration relative to the proximity to the electrical contact. Figure 6 shows non-normalized Cl^- and PO_4^- profiles sampled close to and further away from the electrode following multiple actuation cycles. Overall chloride concentrations are higher closer to the electrode contact point, but phosphate appears to be relatively independent of distance from the contact.

In order to understand these differences in anion concentration, the resistivity of the films was measured at various points in the actuation and at various distances from the electrode contact point. No significant changes in resistivity are observed before vs. after the cure phase at any distance from the contact, indicating that no significant degradation of the film is occurring during this phase. In addition, the resistivity of the film at a point approximately 1 mm away from the contact point did not change significantly even after multiple actuation cycles. However, the resistivity of the film increases at the point closest to the electrode as the number of actuation cycles increases. Specifically, after the first oxidation cycle, resistivity increased at this point to approximately 16 times its original value, and after the fifth oxidation cycle it increased to more than 550 times its original value. These results indicate that there will be a large potential drop in the film as the distance from the contact point increases, explaining the observation that the largest film deflection occurs at the point nearest to the electrical contact as well as the fact that larger concentrations of chlorine are

observed there. The buildup of chlorine itself may be responsible for the increase in resistivity, as a result of overoxidation or other electrochemical effects.

4. Conclusion

In conclusion, a study has been presented of silk/PPy biocomposite bilayer actuators using ToF-SIMS. The composition of the IPN and the profiles of various electrolyte components were explored as a function of the actuation history of the device, depth from the film surface and proximity to the contact electrode. The composition of the biocomposite materials varied as a function of depth in the film, and the ratio of PPy to silk was found to be largest near the surface of the IPN and to decrease steadily through the film, likely due to the more porous nature of the film near the air interface. The concentration of the dopant pTSA during actuation was measured, and it was concluded that dopant leaching did not occur to a significant extent in our devices. Additionally, the changes in concentration of various anions during the actuation process were explored. Both chloride and phosphate enter the devices during the cure phase. Phosphate concentrations do not change appreciably following the cure phase, while chlorine ions accumulate during oxidation more than they leave during reduction, resulting in a steady buildup of chlorine in the IPN during actuation. Finally, ion concentrations and film resistivity were evaluated as a function of distance from the electrical contact, and degradation was found to occur more readily near the contact. The results of this study will be incorporated into the design of next-generation devices to optimize performance.

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References

- [1] E. Smela. Conjugated polymer actuators for biomedical applications, *Adv. Mater. (Weinheim, Ger.)*, 15(6), (2003), 481-494. doi: 10.1002/adma.200390113
- [2] T. Shoa, J.D.W. Madden, N.R. Munce, V. Yang. Analytical modeling of a conducting polymer-driven catheter, *Polym. Int.*, 59(3), (2010) 343-351. doi: 10.1002/pi.2783
- [3] D.D. Ateh, H.A. Navsaria, P. Vadgama. Polypyrrole-based conducting polymers and interactions with biological tissues, *J. R. Soc. Interface*, 3(11), (2006) 741-752. doi: 10.1098/rsif.2006.0141
- [4] H.K.A. Tsai, M. Madou. Microfabrication of bilayer polymer actuator valves for controlled drug delivery, *JALA*, 12(5), (2007) 291-295. doi: 10.1016/j.jala.2007.06.010
- [5] A.S. Hutchison, T.W. Lewis, S.E. Moulton, G.M. Spinks, G.G. Wallace. Development of polypyrrole-based electromechanical actuators, *Synth. Met.*, 113(1-2), (2000) 121-127. doi: 10.1016/S0379-6779(00)00190-9
- [6] M. Pyo, C.C. Bohn, E. Smela, J.R. Reynolds, A.B. Brennan. Direct Strain Measurement of Polypyrrole Actuators Controlled by the Polymer/Gold Interface, *Chem. Mater.*, 15(4), (2003) 916-922. doi: 10.1021/cm020312w
- [7] I.S. Romero, N.P. Bradshaw, J.D. Larson, S.Y. Severt, S.J. Roberts, M.L. Schiller, J.M. Leger, A.R. Murphy. Biocompatible Electromechanical Actuators Composed of Silk-Conducting Polymer Composites, *Adv. Funct. Mater.*, 24(25), (2014) 3866-3873. doi: 10.1002/adfm.201303292
- [8] G.H. Altman, F. Diaz, C. Jakuba, T. Calabro, R.L. Horan, J. Cheng, H. Lu, J. Richmond, D.L. Kaplan. Silk-based biomaterials, *Biomaterials*, 24(3), (2002) 401-416. doi: 10.1016/S0142-9612(02)00353-8

- [9] M.R. Gandhi, P. Murray, G.M. Spinks, G.G. Wallace. Mechanism of electromechanical actuation in polypyrrole, *Synth. Met.*, 73(3), (1995) 247-256. doi: 10.1016/0379-6779(95)80022-0
- [10] M.L. Abel, S.R. Leadley, A.M. Brown, J. Petitjean M.M. Chehimi, J.F. Watts. ToF-SIMS characterization of electrochemically synthesized polypyrrole films, *Synth. Met.*, 66(1), (1994) 85-88. doi: 10.1016/0379-6779(94)90166-X
- [11] E. De Giglio, C.D. Calvano, I. Losito, L. Sabbatini, P.G. Zambonin, A. Torrisi, A. Licciardello. Surface (XPS, SIMS) chemical investigation on poly(pyrrole-3-acetic acid) films electrosynthesized on Ti and TiAlV substrates for the development of new bioactive substrates, *Surf. Interface Anal.*, 37(6), (2005) 580-586. doi: 10.1002/sia.2053
- [12] S.L. Sin, L.L. Teo, K.S. Tan, C.Y. Chan. ESCA and TOF-SIMS study on oxidized and reduced polypyrrole-poly(4-styrene sulfonate) free-standing films synthesized on Ti electrodes, *Electrochem. Commun.*, 2(10), (2000) 685-691. doi: 10.1016/S1388-2481(00)00090-4
- [13] M.L. Abel, M.M. Chehimi, F. Fricker, M. Delamar, A.M. Brown, J.F. Watts. Adsorption of poly(methyl methacrylate) and poly(vinyl chloride) blends onto polypyrrole. Study by X-ray photoelectron spectroscopy, time-of-flight static secondary ion mass spectroscopy, and inverse gas chromatography, *J. Chromatogr. A*, 969(1-2), (2002) 273-285. doi: 10.1016/S0021-9673(02)00894-4
- [14] C. Perruchot, M.M. Chehimi, M. Delamar, J.A. Eccles, T.A. Steele, C.D. Mair. SIMS analysis of conducting polypyrrole-silica gel composites, *Synth. Met.*, 113(1-2), (2000) 53-63. doi: 10.1016/S0379-6779(99)00303-3
- [15] R. Brooke, M. Fabretto, P. Hojati-Talemi, P. Murphy, D. Evans. Evidence for 'bottom up' growth during vapor phase polymerization of conducting polymers, *Polymer*, 55(16), (2014) 3458-3460. doi: 10.1016/j.polymer.2014.06.055
- [16] N. Wehbe, T. Mouhib, A. Delcorte, P. Bertrand, R. Moellers, E. Niehlus, L. Houssiau. Comparison of fullerene and large argon clusters for the molecular depth profiling of amino acid multilayers, *Anal. Bioanal. Chem.*, 406(1), (2014) 201-211. doi: 10.1007/s00216-013-7408-x
- [17] J.S. Fletcher, X.A. Conlan, N.P. Lockyer, J.C. Vickerman. Molecular depth profiling of organic and biological materials, *Appl. Surf. Sci.*, 252(19), (2006) 6513-6516. doi: 10.1016/j.apsusc.2006.02.213
- [18] Y. Liu, C. Lu, S. Twigg, M. Ghaffari, J. Lin, N. Winograd, Q.M. Zhang. Direct observation of ion distributions near electrodes in ionic polymer actuators containing ionic liquids, *Sci. Rep.*, 3, (2013) 973-977. doi: 10.1038/srep00973

- [19] S. K. Mahadeva, S. Yun, J. Kim. Flexible humidity and temperature sensor based on cellulose–polypyrrole nanocomposite, *Sens. Actuators. A: Physical* 165(2), 194-199, 2011.
- [20] S. K. Mahadeva, J. Kim. Nanocoating of ionic liquid and polypyrrole for durable electro-active paper actuators working under ambient conditions, *J. Phys. D: Appl. Phys.* 43 (2010), 205502. doi:10.1088/0022-3727/43/20/205502
- [21] M. Holzweber, A.G. Shard, H. Jungnickel, A. Luch, W.E.S. Unger. Dual beam organic depth profiling using large argon cluster ion beams, *Surf. Interface Anal.*, 46(10-11), (2014) 936-939. doi: 10.1002/sia.5429
- [22] S. Rabbani, A.M. Barber, J.S. Fletcher, N.P. Lockyer, J.C. Vickerman. TOF-SIMS with Argon Gas Cluster Ion Beams: A Comparison with C60+, *Anal. Chem. (Washington, DC, U. S.)*, 83(10), (2011) 3793-3800. doi: 10.1021/ac200288v
- [23] A.G. Shard, R. Havelund, M.P. Seah, S.J. Spencer, I.S. Gilmore, N. Winograd, D. Mao, T. Miyayama, E. Niehuis, D. Rading, R. Moellers. Argon Cluster Ion Beams for Organic Depth Profiling: Results from a VAMAS Interlaboratory Study, *Anal. Chem. (Washington, DC, U. S.)*, 84(18), (2012) 7865-7873. doi: 10.1021/ac301567t
- [24] P. Chiarelli, A. Della Santa, D. De Rossi, A. Mazzoldi. Actuation properties of electrochemically driven polypyrrole free-standing films, *J. Intell. Mater. Syst. Struct.*, 6(1), (1995) 32-37. doi: 10.1177/1045389X9500600105
- [25] C. Baiocchi, M.C. Brussino, E. Pramauro, A.B. Prevot, L. Palmisano, G. Marci. Characterization of methyl orange and its photocatalytic degradation products by HPLC/UV-VIS diode array and atmospheric pressure ionization quadrupole ion trap mass spectrometry, *Int. J. Mass Spectrom.*, 214(2), (2002) 247-256. doi: 10.1016/S1387-3806(01)00590-5
- [26] A. Bianco Prevot, D. Fabbri, E. Pramauro, C. Baiocchi, C. Medana. High-performance liquid chromatography coupled to ultraviolet diode array detection and electrospray ionization mass spectrometry for the analysis of intermediates produced in the initial steps of the photocatalytic degradation of sulfonated azo dyes, *J. Chromatogr. A*, 1202(2), (2008) 145-154. doi: 10.1016/j.chroma.2008.06.038

Figures

Figure 1: Representative SEM micrograph of the cross-section of a bilayer actuator film where the thickness of the silk-PPy IPN and the underlying unmodified silk support are highlighted. To facilitate formation of the IPN during pyrrole polymerization, anionic sulfonic acid groups were covalently attached to the tyrosine residues of silk as shown.

Figure 2: Non-corrected H⁻ (triangle), CN⁻ (circle), and CNO⁻ (square) signal intensities from a non-actuated film.

Figure 3: Corrected and normalized ion signal intensities for CN^- (solid squares), CNO^- (solid circles), C_2^- (open squares) and benzenesulfonic acid radical (open circles) from a non-actuated film.

Figure 4: Corrected pTSA anion signal intensities for a non-actuated film (solid square), a film after the cure phase (open square), after the eighth reduction (solid triangle) and after the eighth oxidation (open triangle).

Figure 5: Corrected anion signal intensities for A) Cl^- and B) PO_4^{3-} from a non-actuated film (solid square), a film after the cure phase (open square), following first reduction (solid circle), second reduction (open circle), and second oxidation (solid triangle).

Figure 6: Cl^- (top) and PO_4^{3-} (bottom) signals from a sample that has undergone a cure, seven full actuation cycles and a reduction. Characterization was performed at the point on the strip closest to the electrode (solid line) and 3.1 mm below the first sample site (dashed line).